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Multi-residue Screening of Veterinary Drugs (I) and (II) in Meat According to the Japan Positive List using Cartridge-based SPE and LC/MS/MS

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Introduction

On May 29, 2006, Japan's Ministry of Health, Labour, and Welfare (MHLW), equivalent to the FDA in the US, introduced the Japanese Positive List for detection of agricultural chemicals in foods[1]. The legislation was developed to prohibit the distribution of chemically contaminated foods that contain agricultural chemicals above a maximum residue limit (MRL). The agricultural chemicals include pesticides, feed additives, and veterinary drugs. The regulations apply to all domestically produced and imported foodstuffs and comprise a list of almost 800 chemicals. As Japan is the biggest importer in the Western Pacific/Australasia region, most food exporting countries are obliged to follow its guidelines. The regulation's testing protocols use classic cartridge-based SPE and LC/MS or GC/MS techniques, and require that no agricultural chemical exceed the MRL (typically 0.01 ppm).

This note describes the analysis of 45 neutral, basic, and acidic veterinary drugs in different meat matrices down to the MRLs (0.01 ppm) of the Japanese Positive List.

By using Hydromatrix™ diatomaceous earth and Bond Elut™ Plexa™ SPE cartridges, Pursuit™ C18 HPLC columns and 320-MS detection, Varian offers a complete solution for the detection of veterinary drugs in meat.

Materials and methods

Matrices: Chicken, pork, and beef.
Methodology: For ease of detection and separation, the drugs were screened in two groups. Group 1 comprised predominantly basic compounds, and group II covered acidic and basic compounds. The groups were analyzed using different LC and MS/MS conditions (Method (I) and Method (II), Table 1), but with identical sample preparation steps.

Table 1. Analytes used for both Methods (I) and (II).

Method (I)	Method (II)
5-Propylsulfonyl-1H-benzimidazole-2-amine	Lincomycin
Thiabendazole	Sulfacetamide
Levamisole	Danofloxacin
Sulfadiazine	Xylazine
Sulfathiazole	Clenbuterol
Trimethoprim	Trichlorfon (DEP)
Sulfapyridine	Tilmicosin
Ormetoprim	Pyrimethamine
Sulfamerazine	Florfenicol
Thiamphenicol	2-Acetylamino-5-nitrothiazole
Sulfadimidine	Clorsulon
Sulfamethoxypyridazine	Prednisolone
Sulfamonomethoxine	Hydrocortisone
Sulfachlorpyridazine	Tiamulin
Sulfadoxine	Dexamethasone
Sulfamethoxazole	Famphur
Ethopabate	Fenobucarb (BPMC)
Sulfaquinoxaline	Emamectin Bla
Sulfadimethoxine	Temephos (Abate)
Sulfanitran	Allethrin
beta-Trenbolone	Monensin
alpha-Trenbolone	
Melengestrol acetate	
Zeranol	

Instrumentation

Detector: Varian 320-MS LS/MS/MS
LC Pumps: Varian 212

Materials and Reagents

Filter: Hydromatrix (Part no. 198003)
SPE Cartridge: Bond Elut Plexa 3 mL, 60 mg (Part no. 12109603)
HPLC Column: Pursuit C18 3 µm, 150 x 3.0 mm (Part no. A3001150X030)

Standards: Drug mixture solutions, PL-2-1 and PL-1-3 for basic, acidic, and neutral analytes (Wako, Japan)
Concentration: 20 µg/mL of each in methanol

Sample preparation for both methods

1. Weigh 5 g of the meat sample.
2. Add 100 mL of acetonitrile/methanol/0.2% meta-phosphoric acid (1:1:3) and homogenize. Filter under vacuum with filter paper with a 2-3 mm thick layer of Hydromatrix™ diatomaceous earth. Rinse with 20 mL ACN/methanol/meta phosphoric acid (1:1:3). Filter under vacuum with filter paper containing diatomaceous earth.
3. Pool filtrates and evaporate to 20 mL.
4. Condition Bond Elut Plexa™ with 5 mL methanol and 5 mL 2% ammonium hydroxide, load sample and wash with 5 mL 2% ammonium hydroxide, then elute sample with 5 mL methanol.
5. Evaporate to dryness at 40 °C.
6. Re-dissolve sample with 1 mL 1:9 ACN:water.

LC protocol for Method (I)

Column: Pursuit™ C18 3 µm, 150 x 3.0 mm
Mobile Phase A: ACN + 0.1% Formic Acid
Mobile Phase B: H2O + 0.1% Formic Acid
Gradient:

Time (min)	%A	%B	Flow (µL/min)
0	5	95	200
2	5	95	200
30	80	20	200
34	80	20	200
35	5	95	200
40	5	95	200

Column Temp: 40 °C

MS protocol for Method (I)

Manifold Temp: 40 °C
API Housing Temp: 65 °C
API Drying Gas Program: 400 °C for 19 min, down to 300 °C in 1 min, keep at 300 °C for 15 min

Table 2. MS/MS transition details for Method (I).

Compound	Parent Ion	Daughter Ion	ESI Mode	Collision Energy
5-Propylsulfonyl-1H-benzimidazole-2-amine	240.1	133.1	(+)	21.5
Thiabendazole	202.1	175.1	(+)	8.0
Levamisole	205.1	178.0	(+)	16.0
Sulfadiazine	251.1	92.1	(+)	15.0
Sulfathiazole	256.0	156.0	(+)	6.0
Trimethoprim	291.1	230.1	(+)	10.5
Sulfapyridine	250.0	156.2	(+)	13.5
Ormetoprim	275.1	123.1	(+)	14.0
Sulfamerazine	265.1	155.9	(+)	11.5
Thiamphenicol	356.0	308.0	(+)	8.0
Sulfadimidine	279.1	92.1	(+)	19.0
Sulfamethoxypyridazine	281.1	156.1	(+)	14.5
Sulfamonomethoxine	281.1	156.1	(+)	14.5
Sulfachlorpyridazine	286.1	157.1	(+)	8.0
Sulfadoxine	311.1	156.2	(+)	17.5
Sulfadimethoxine	311.1	156.2	(+)	17.5
Sulfamethoxazole	254.0	155.9	(+)	12.0
Ethopabate	238.2	136.0	(+)	15.0
Sulfaquinoxaline	301.0	155.9	(+)	12.0
Sulfanitran	336.1	134.1	(+)	19.5
beta-Trenbolone	271.1	165.1	(+)	38.0
alpha-Trenbolone	271.1	165.1	(+)	38.0
Melengestrol Acetate	397.2	279.1	(+)	14.0
Zeranol	321.1	277.1	(+)	21.0

LC protocol for Method (II)

Column: Pursuit C18 3 µm, 150 x 3.0 mm
Mobile Phase A: ACN + 0.1% Formic Acid
Mobile Phase B: H_2O, 0.1% Formic Acid
Gradient:

Time (min)	%A	%B	Flow (µL/min)
0	5	95	200
28	99	1	200
33	99	1	200
34	5	95	200
40	5	95	200

Column Temp: 40 °C

MS protocol for Method (II)

Manifold Temp: 40 °C
API Housing Temp: 65 °C
API Drying Gas Program: 275 °C for 19 min, up to 400 °C in 1.25 min, keep at 400 °C for 16 min

Table 3. MS/MS transition details for Method (II).

Compound	Parent Ion	Daughter Ion	ESI Mode	Collision Energy
Lincomycin	407.2	126.1	(+)	19.5
Sulfacetamide	215.0	156.0	(+)	11.0
Danofloxacin	358.1	340.1	(+)	18.5
Xylazine	221.1	90.1	(+)	10.5
Clenbuterol	278.2	204.1	(+)	9.5
Trichlorfon (DEP)	259.0	109.1	(+)	9.5
Tilmicosin	435.6	143.0	(+)	14.0
Pyrimethamine	249.1	177.1	(+)	19.0
Florfenicol	355.9	184.9	(-)	18.0
2-Acetylamino-5-nitrothiazole	186.0	138.9	(-)	13.0
Clorsulon	379.9	343.9	(-)	10.5
Famphur	326.0	93.1	(+)	20.5
Hydrocortisone	363.1	121.1	(+)	14.5
Tiamulin	494.3	192.1	(+)	12.0
Dexamethasone	393.2	373.1	(+)	5.0
Prednisolone	361.2	147.0	(+)	13.0
Fenobucarb (BPMC)	208.1	95.1	(+)	10.5
Emamectin Bla	887.5	158.1	(+)	19.0
Temephos (Abate)	467.0	419.3	(+)	19.5
Allethrin	303.0	135.0	(+)	7.0
Monensin	693.4	675.2	(+)	30.5

Results and Discussion

A standard method for analyzing veterinary drugs in animal products in accordance with the Japanese Positive List has not been published. However, a government recommended test method does exist, which was followed with some modifications to give Method (I) and Method (II). Sample preparation with Hydromatrix™, followed by Bond Elut Plexa™ SPE, can effectively clean up the matrix interferences. A diatomaceous earth product such as Hydromatrix is necessary to remove turbidity when meat samples are treated with a mixture of acetonitrile/methanol/meta phosphoric acid and they undergo protein denaturation. Bond Elut Plexa was conditioned with methanol and 2% ammonium hydroxide, a deviation from the normal procedure that uses methanol and water, as it was found that the recoveries were higher when ammonium hydroxide was used.

MRM transitions used for most compounds in both methods were based on information provided in the Certificate of Analysis by the supplier of the standards, but with monitoring ions changed in a few cases to achieve better sensitivity. Tables 2 and 3 list the MS/MS transition details of all analytes used. A temperature program for drying gas was included in both methods as some of the compounds were not stable at high temperatures.

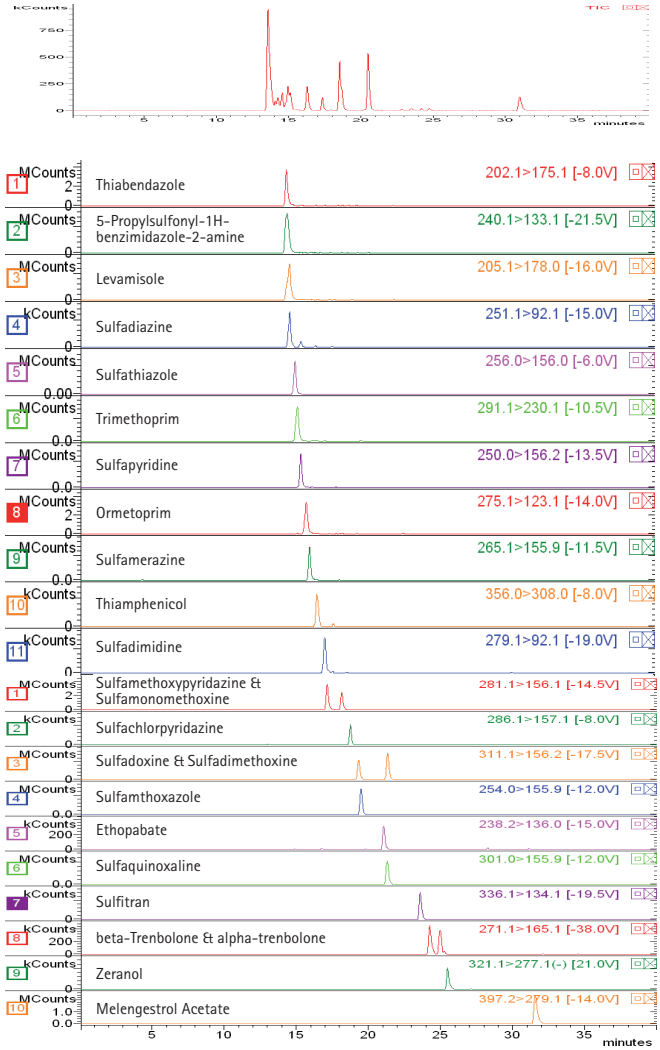
kCounts
TIC BPC
minutes
1 Thiabendazole 202.1>175.1 [-8.0V]
2 5-Propylsulfonyl-1H-benzimidazole-2-amine 240.1>133.1 [-21.5V]
3 Levamisole 205.1>178.0 [-16.0V]
4 Sulfadiazine 251.1>92.1 [-15.0V]
5 Sulfathiazole 256.0>156.0 [-6.0V]
6 Trimethoprim 291.1>230.1 [-10.5V]
7 Sulfapyridine 250.0>156.2 [-13.5V]
8 Ormetoprim 275.1>123.1 [-14.0V]
9 Sulfamerazine 265.1>155.9 [-11.5V]
10 Thiamphenicol 356.0>308.0 [-8.0V]
11 Sulfadimidine 279.1>92.1 [-19.0V]
1 Sulfamethoxypyridazine & Sulfamonomethoxine 281.1>156.1 [-14.5V]
2 Sulfachlorpyridazine 286.1>157.1 [-8.0V]
3 Sulfadoxine & Sulfadimethoxine 311.1>156.2 [-17.5V]
4 Sulfamthoxazole 254.0>155.9 [-12.0V]
5 Ethopabate 238.2>136.0 [-15.0V]
6 Sulfaquinoxaline 301.0>155.9 [-12.0V]
7 Sulfitran 336.1>134.1 [-19.5V]
8 beta-Trenbolone & alpha-trenbolone 271.1>165.1 [-38.0V]
9 Zeranol 321.1>277.1(-) [21.0V]
10 Melengestrol Acetate 397.2>279.1 [-14.0V]

Figure 1. LC/MS/MS analysis of Method (I) standards on Pursuit C18 showing total ion chromatogram and MRM chromatograms.

Figures 1 and 3 show LC/MS/MS analysis of Method (I) and (II) standards, with total ion and MRM chromatograms. There were three pairs of isomers in Method (I); each pair was resolved with base-line resolution on Pursuit™ C18, demonstrating the power of LC when MS detection becomes a limitation. The same column was used for both methods. Overall, it appears to be well suited for multi-residue veterinary drug analysis.

Figures 2 and 4 are total ion chromatograms of Method (I) and (II) compounds in standard and spiked matrices (chicken, pork, and beef) at 10 ppb. At 10 ppb, differences between each type of meat matrix after clean-up are fairly small, indicative of the excellent clean-up by Bond Elut Plexa. Tables 4 and 5 list recoveries and RSD values for all analytes covered in both methods from chicken, pork, and beef with Bond Elut

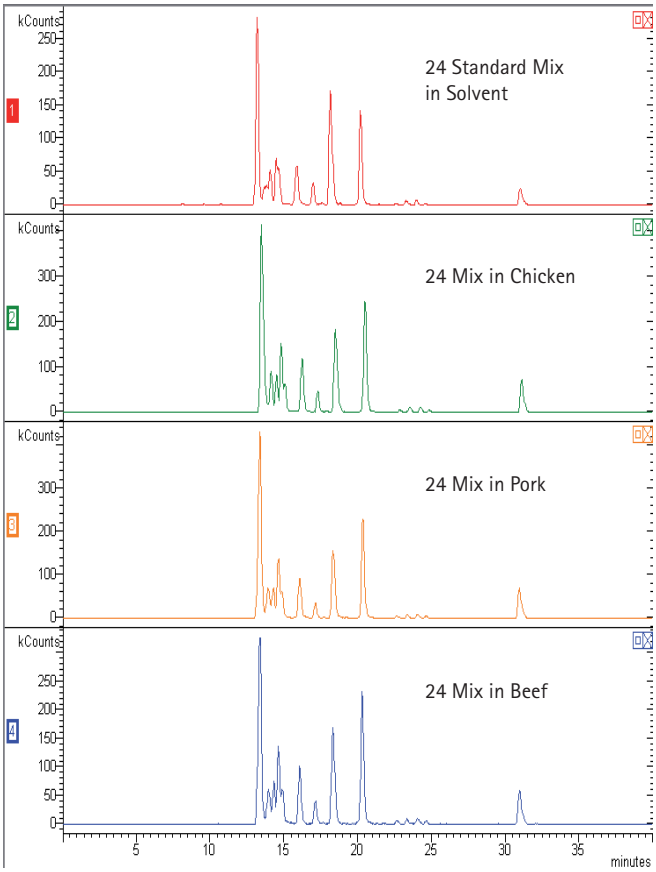

Figure 2. Total ion chromatograms of Method (I) compounds in standard and spiked matrices (chicken, pork, and beef) at 10 ppb for quantitation.

Plexa™ at 10 ppb. Reproducibility was impressive, as RSD values for most compounds was within 1%, the maximum being 3.6%. Recoveries for all drugs were in the range of 65% - 115%, most falling within 75% - 100%. These recoveries are within the EU and CDFA requirements[2].

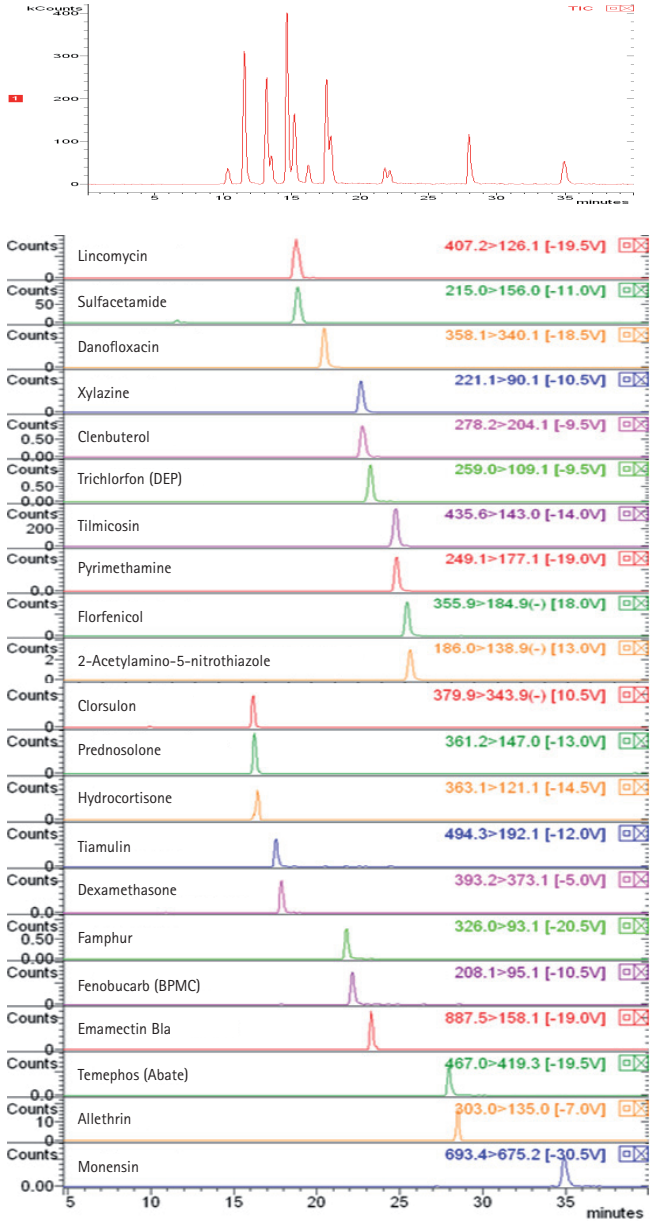

Figure 3. LC/MS/MS analysis of Method (II) standards on Pursuit™ C18 showing total ion chromatogram and MRM chromatograms.

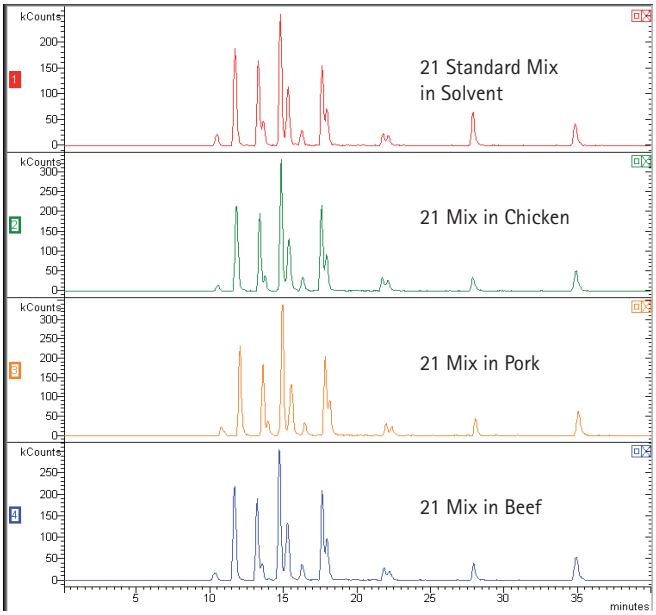

Figure 4. Total ion chromatograms of Method (II) compounds in standard and spiked matrices (chicken, pork, and beef) at 10 ppb for quantitation.

Table 4. Method (I): multi-suite extraction of veterinary drugs from chicken, pork, and beef with Bond Elut Plexa™ and recoveries at 10 ppb detection limits.

Compound	Chicken			Pork			Beef		
	C (ppb)	RSD	Recovery (%)	C (ppb)	RSD	Recovery (%)	C (ppb)	RSD	Recovery (%)
5-Propylsulfonyl-1H-benzimidazole-2-amine	10.0	0.8	100	8.4	0.5	84	8.6	0.2	86
Thiabendazole	11.5	0.3	115	8.6	0.5	86	10.1	0.9	101
Levamisole	7.5	0.2	75	6.5	0.6	65	6.5	0.6	65
Sulfadiazine	10.8	1.0	108	9.6	0.4	96	10.0	0.1	100
Sulfathiazole	7.8	0.3	78	7.8	0.9	78	7.0	0.5	70
Trimethoprim	8.7	0.2	87	7.0	0.3	70	7.5	0.3	75
Sulfapyridine	8.4	0.0	84	8.5	0.2	85	8.1	0.5	81
Ormetoprim	9.1	0.1	91	7.6	0.1	76	7.9	0.7	79
Sulfamerazine	8.7	0.7	87	8.7	0.3	87	8.5	0.2	85
Thiamphenicol	11.1	1.4	111	7.9	3.5	79	9.6	3.3	96
Sulfadimidine	9.6	1.9	96	9.0	0.9	90	8.5	0.8	85
Sulfamethoxypyridazine	8.9	0.2	89	8.3	0.2	83	8.1	0.2	81
Sulfamonomethoxine	9.9	1.1	99	9.8	0.8	98	8.9	1.0	89
Sulfachlorpyridazine	10.0	0.7	100	8.4	0.6	84	9.6	1.4	96
Sulfadoxine	10.3	1.0	103	9.4	0.2	94	9.2	0.4	92
Sulfamethoxazole	10.1	0.5	101	8.6	1.0	86	8.8	0.6	88
Ethopabate	11.2	3.6	112	11.3	2.0	113	10.0	0.4	100
Sulfaquinoxaline	11.5	0.5	115	10.3	0.2	103	10.0	0.2	100
Sulfadimethoxine	9.6	0.7	96	10.1	0.5	101	9.5	0.6	95
Sulfanitran	10.7	0.8	107	11.3	0.5	113	10.7	0.3	107
beta-Trenbolone	10.7	1.5	107	9.7	1.8	97	9.0	0.9	90
alpha-Trenbolone	9.8	0.1	98	8.5	0.0	85	8.6	0.9	86
Melengestrol acetate	9.7	0.2	97	8.5	1.2	85	7.9	0.6	79
Zeranol	9.7	2.1	97	9.4	0.3	94	9.1	0.2	91

Table 5. Method (II): multi-suite extraction of veterinary drugs from chicken, pork, and beef with Bond Elut Plexa™ and recoveries at 10 ppb detection limits.

Compound	Chicken			Pork			Beef		
	C (ppb)	RSD	Recovery (%)	C (ppb)	RSD	Recovery (%)	C (ppb)	RSD	Recovery (%)
Lincomycin	8.8	1.5	88	7.3	1.6	73	9.7	0.5	97
Sulfacetamide	9.4	0.9	94	8.7	0.9	87	8.9	0.9	89
Danofloxacin	11.3	1.5	113	10.2	1.5	102	10.5	1.1	105
Xylazine	10.8	0.5	108	10.6	1.3	106	9.6	0.8	96
Clenbuterol	9.4	1.2	94	9.7	0.2	97	9.6	0.1	96
Trichlorfon (DEP)	8.4	0.4	84	7.2	0.2	72	7.5	0.2	75
Tilmicosin	9.3	1.0	93	9.4	1.3	94	9.1	0.9	91
Pyrimethamine	10.0	0.9	100	10.1	0.3	101	9.8	0.5	98
Florfenicol	10.6	0.1	106	9.9	0.5	99	10.3	0.5	103
2-Acetylamino-5-nitrothiazole	9.5	0.5	95	9.8	1.0	98	9.7	0.7	97
Clorsulon	9.8	1.1	98	10.0	0.2	100	9.6	0.3	96
Prednisolone	10.2	1.0	102	9.9	1.0	99	9.9	0.6	99
Hydrocortisone	9.4	1.2	94	9.7	1.9	97	8.2	2.0	82
Tiamulin	10.9	2.4	109	9.3	0.4	93	9.8	0.7	98
Dexamethasone	11.1	2.1	111	9.3	0.6	93	9.9	0.3	99
Fenobucarb (BPMC)	11.3	3.3	113	8.5	0.7	85	9.9	0.6	99
Emamectin Bla	7.1	2.3	71	8.7	1.7	87	10.5	1.8	105
Temephos (Abate)	9.1	1.3	91	9.1	1.3	91	9.8	0.2	98
Famphur	10.3	1.4	103	10.0	1.2	100	9.9	0.4	99
Allethrin	10.8	1.5	108	9.6	2.2	96	9.2	1.2	92
Monensin	10.3	1.5	103	9.5	0.2	95	10.1	1.0	101

Conclusions

A complete solutions package of SPE and HPLC products for multi-residue screening of challenging veterinary drugs within the expected MRLs (0.01 ppm) of the Japanese Positive List in a number of meat matrices was developed. Meat matrices investigated included chicken, pork, and beef.

Forty-five compounds were analyzed by two methods using cartridge-based SPE and LC/MS/MS. Hydromatrix™ was used as a filter to remove turbidity. Bond Elut Plexa™ clean-up delivered good reproducibility, with RSD values for most compounds within 1%. Recoveries for both methods were also good, with most falling between 75% and 100%.

The Pursuit C18™ column separated three pairs of isomers with base-line resolution in Method (I), illustrating the power of liquid chromatography when MS detection becomes a limitation. Pursuit C18 is suitable for multi-residue veterinary drugs analysis.

The analytical methods developed met or exceeded the 10 ppb detection limit requirement set by the Japanese Positive List. Both methods were sensitive, reliable, and cost effective.

References

1. Positive List System for Agricultural Chemical Residues in Foods. http://www.ffcr.or.jp.
2. Quality Control Procedures for Pesticide Residues Analysis. SANCO/10232/2006, 24 March 2006. http://ec.europa.eu/food/plant/resources/qualcontrol_en.pdf

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